MYC-MEDIATED TRANSCRIPTIONAL REGULATION OF MITOCHONDRIAL CHAPERONE TRAP1 CONTROLS PRIMARY AND METASTATIC TUMOR GROWTH

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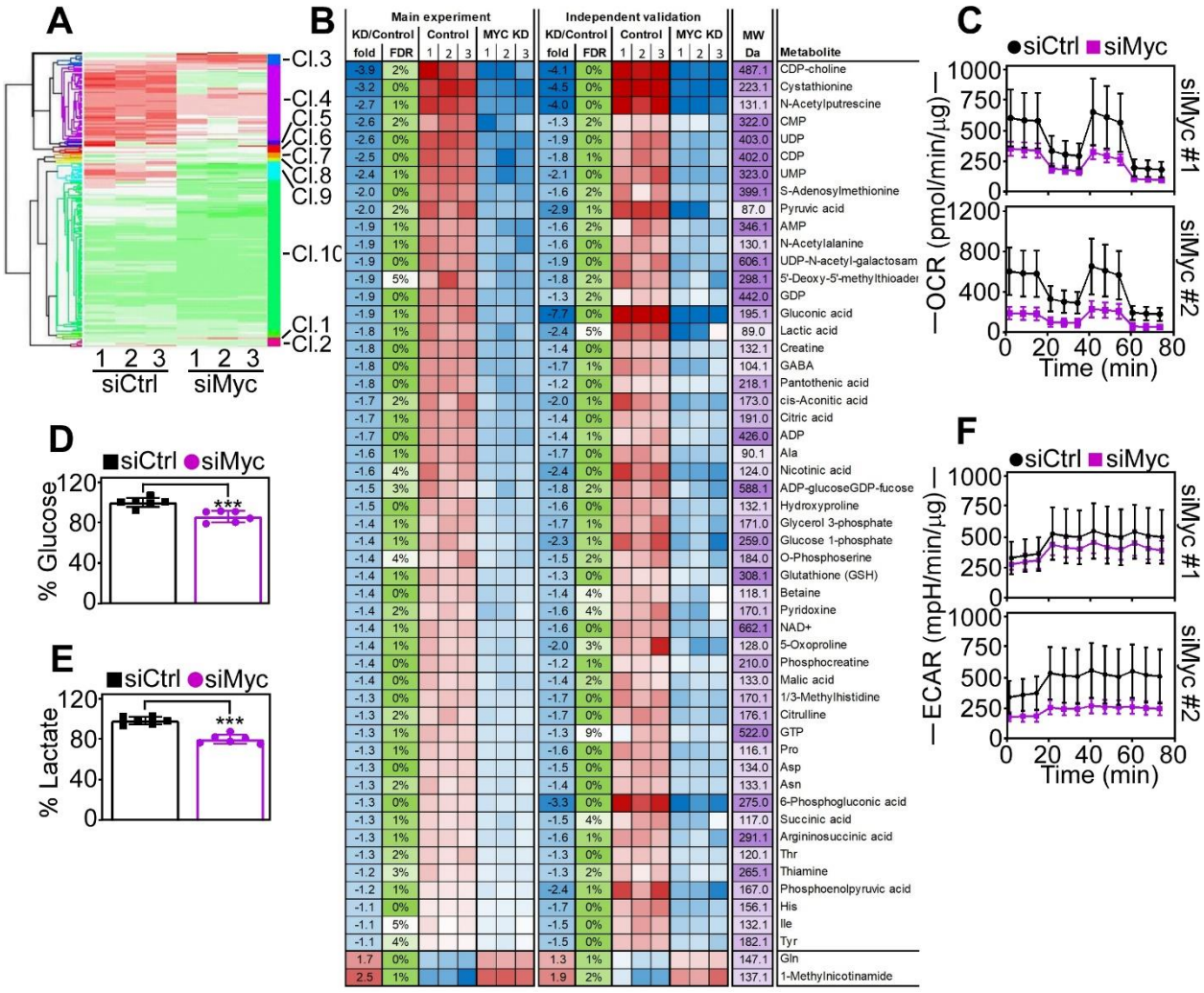
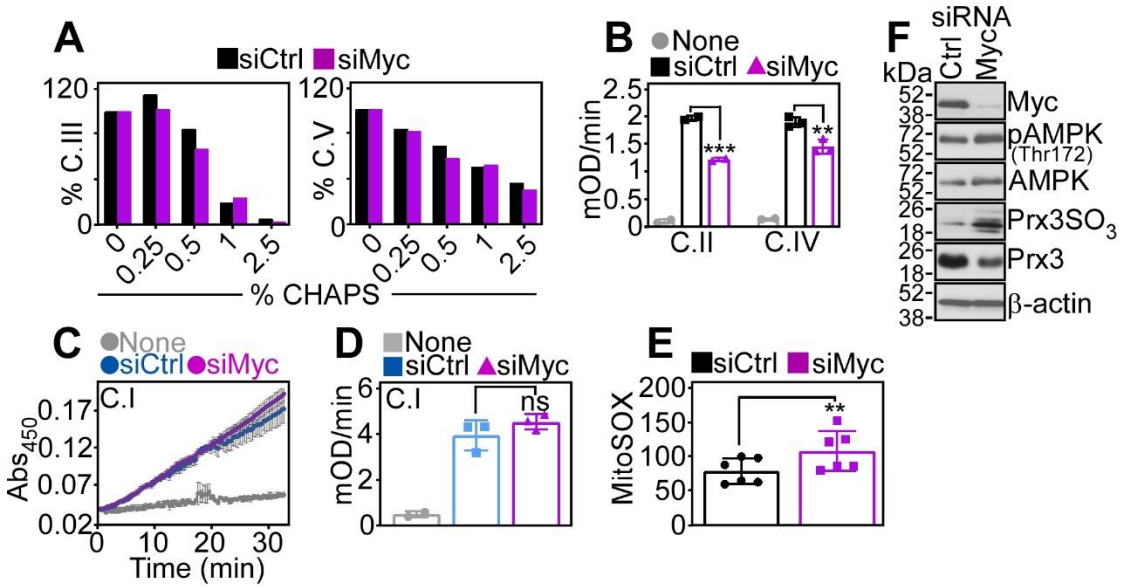
SUPPLEMENTARY MATERIAL

SUPPLEMENTARY FIGURE LEGENDS

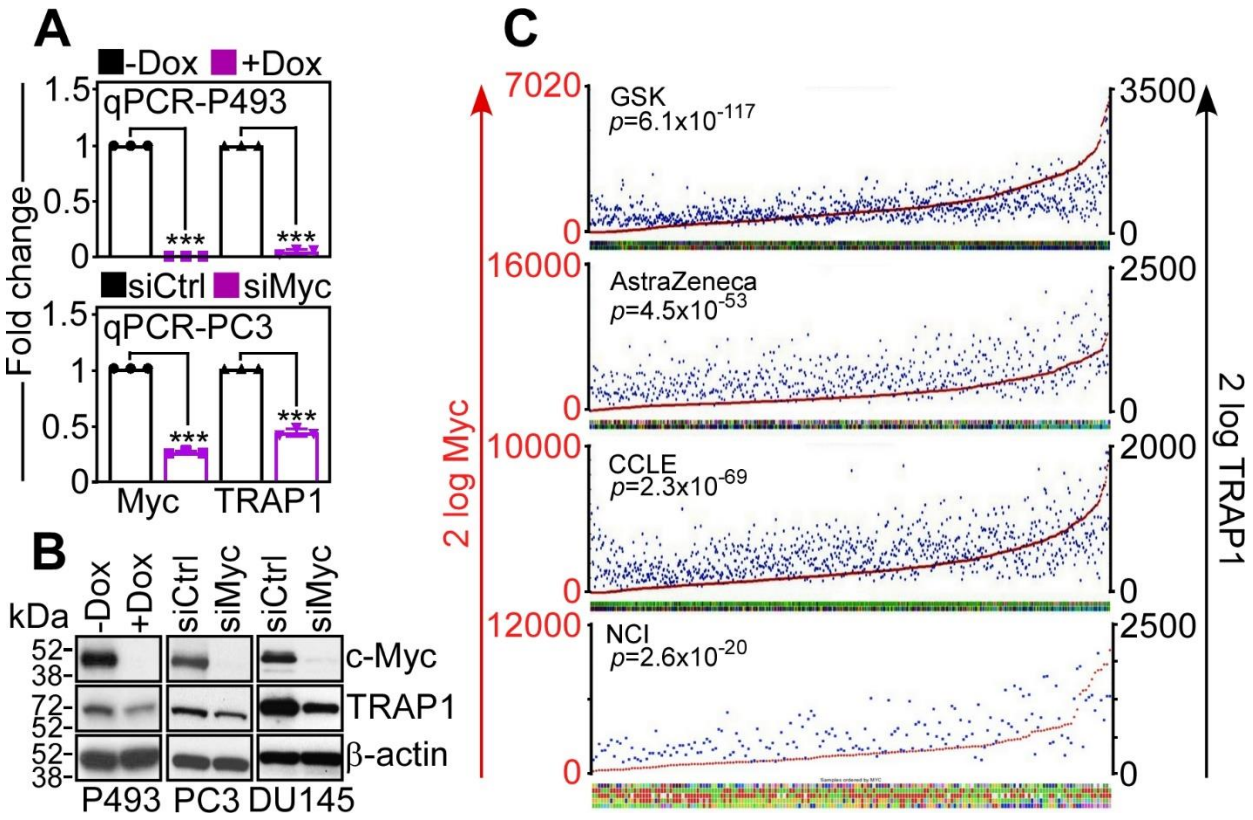


Supplementary Figure S1. Myc regulation of tumor metabolism. *A*, Heat map of changes in metabolite levels in PC3 cells transfected with control non-targeting siRNA (siCtrl) or Myc-directed siRNA (siMyc) (3 replicates per condition). Cl, cluster. *B*, Heat map of metabolites significantly changed in PC3 cells transfected with control siRNA (Control) or Myc-directed siRNA (Myc KD). Only metabolites that overlapped between two studies of global metabolomics profiling (~5,000 biochemicals) and focused metabolite analysis (~300 biochemicals) are shown. Each experiment was done in triplicate. For each metabolite, the fold change and false discovery rate (FDR) are indicated. *C*, PC3 cells transfected with siCtrl or two

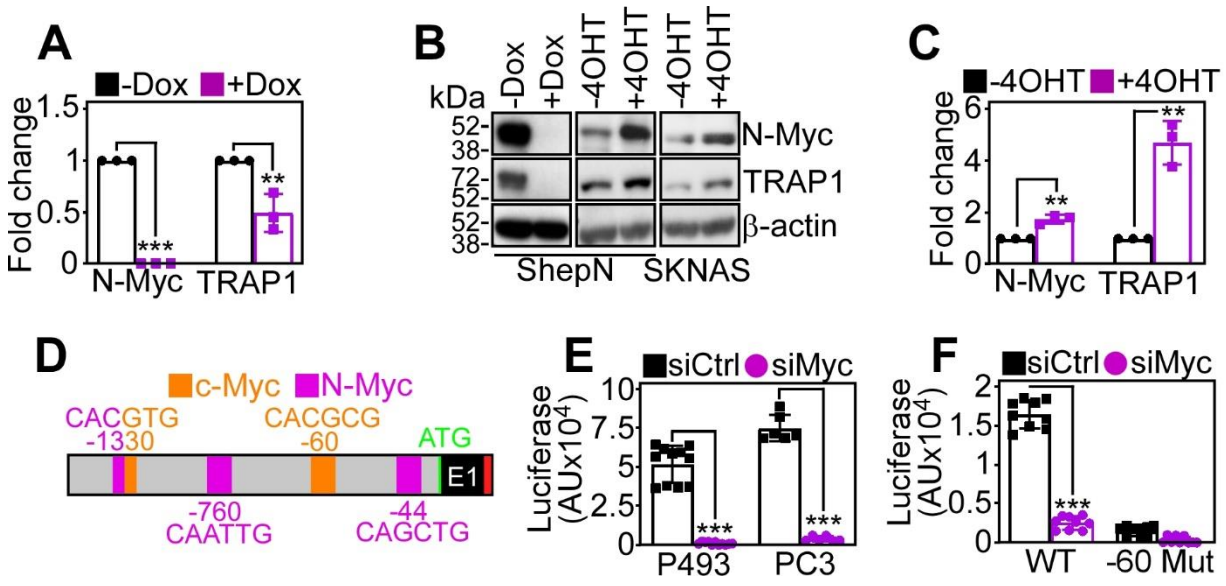
independent Myc-directed siRNA (siMyc #1 and siMyc #2) were analyzed for oxygen consumption rates (OCR) on a Seahorse XFe96 Bioenergetics Flux Analyzer. Representative tracings (n=3). *D and E,* The conditions are as in (*A*) and transfected PC3 cells were analyzed for glucose consumption (*D*) or lactate production (*E*). Mean±SD (n=7-6). ***, p=0.0008 - <0.0001. *F,* The conditions are as in (C) and transfected PC3 cells were analyzed for ExtraCellular Acidification Rates on Seahorse XFe96 Bioenergetics Flux Analyzer. Representative tracings (n=3).



Supplementary Figure S2. Control of mitochondrial protein folding and oxidative bioenergetics by Myc. *A*, PC3 cells were transfected with siCtrl or siMyc and detergent (CHAPS)-insoluble proteins bands corresponding to oxidative phosphorylation complex subunit ubiquinol-cytochrome c reductase core protein 2 (UQCRC2, Complex III, left panel) or ATP synthase F1 subunit (ATP5A, Complex V, right panel) were quantified by densitometry. Representative experiment. *B,* PC3 cells transfected as in (*A*) were analyzed for citrate synthase-normalized mitochondrial Complex (C) II or C. IV activity. Mean±SD (n=3). **, p=0.007; ***, p<0.0001. *C and D,* PC3 cells transfected as in (*A*) were analyzed for mitochondrial oxidative phosphorylation C. I function (*C*, representative tracings) and citrate synthase-normalized activity was quantified (*D*). Mean±SD (n=3). ns, not significant. *E and F,* PC3 cells transfected as in (*A*) were analyzed for mitochondrial superoxide production by MitoSOX staining and flow cytometry (*E*) or Western blotting (*F*). Mean±SD (n=6). ***, p<0.0001. p, phosphorylated.

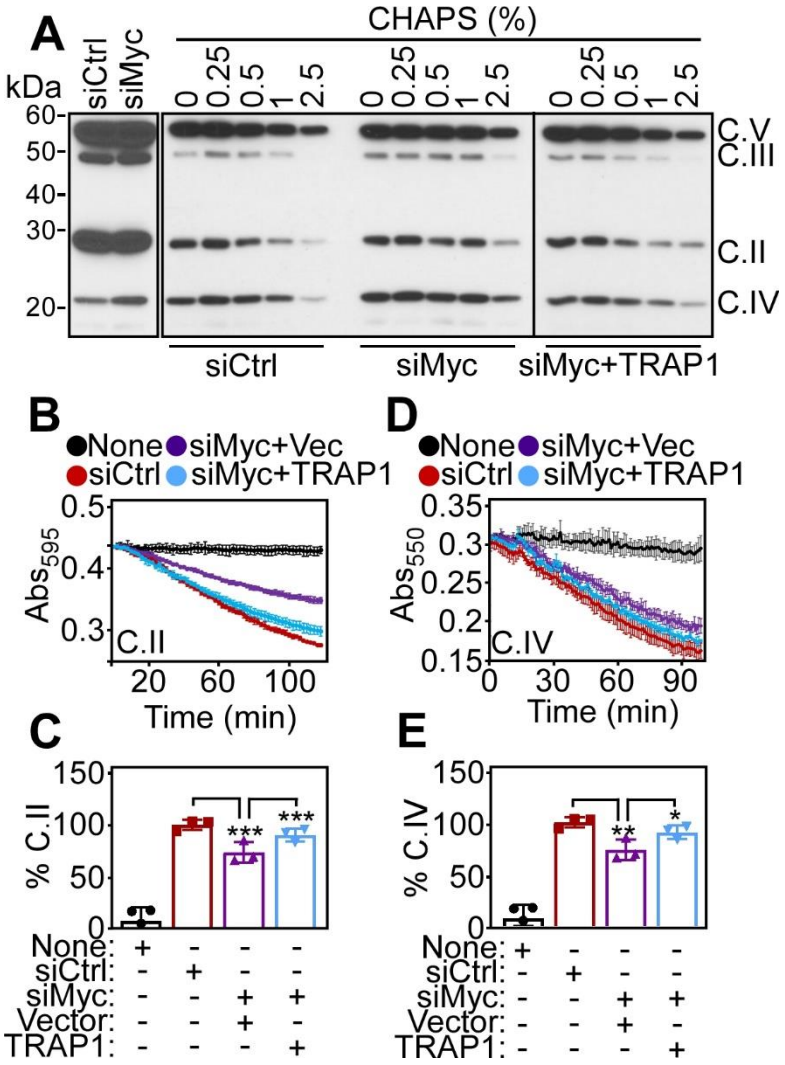


Supplementary Figure S3. Myc transcriptional control of TRAP1. *A*, P493 (top panel) or PC3 (bottom panel) cells were analyzed for changes in expression of Myc or TRAP1 mRNA in the presence (+) or absence (-) of Dox (top) or after transfection with control siRNA or Myc-directed siRNA (bottom), by quantitative PCR. Mean±SD (n=3). ***, p<0.0001. *B*, P493, PC3 or DU145 cells incubated with or without Dox (P493) or transfected with control siCtrl or siMyc (PC3, DU145) were analyzed by Western blotting. *C*, Correlation between Myc and TRAP1 mRNA expression in four cancer cell line databases, Glaxo-Smith-Kline, GSK (n=950), AstraZeneca (n=627), Cancer Cell Line Encyclopedia, CCLE (n=917), and NCI (n=60). Individual p values are indicated.



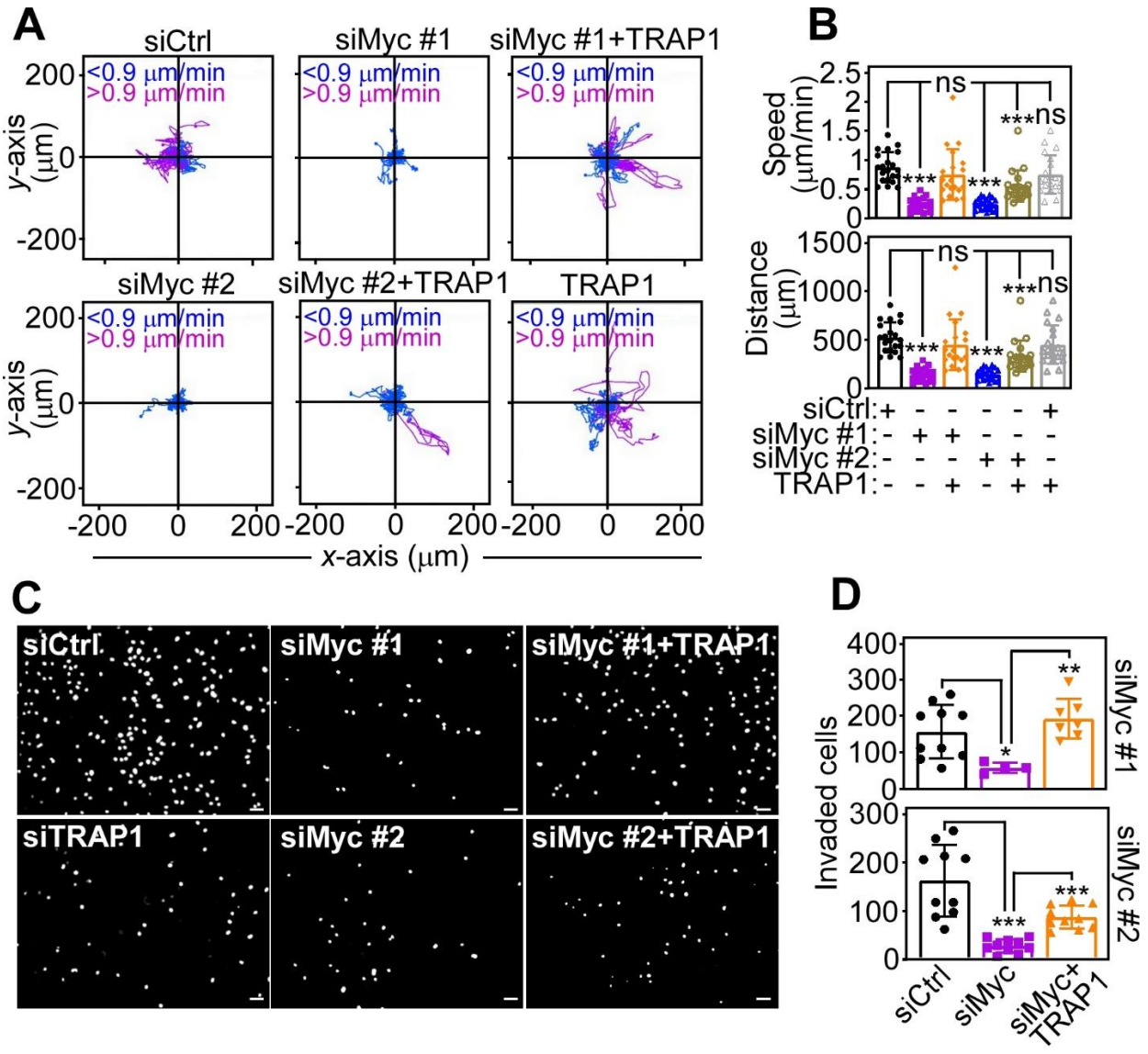
Supplementary Figure S4. N-Myc regulation of TRAP1. *A,* Neuroblastoma Shep21 cells expressing a Dox-regulated inducible Myc transgene (Dox-off) were analyzed for changes in N-Myc or TRAP1 mRNA expression in the presence (+) or absence (-) of Dox, by quantitative PCR (qPCR). Mean±SD (n=3). **, p=0.001; ***, p<0.0001. *B,* Neuroblastoma SHEP or SKNAS cells containing a Dox- or 4OHT-regulated Myc transgene, respectively, were analyzed after conditional Myc silencing (+Dox) or Myc induction (+4OHT), by Western blotting. Individual gels for ShepN and SKNAS cell extracts were examined. *C,* Neuroblastoma SKNAS cells expressing a 4OHT-regulated inducible N-Myc transgene as in (*B*) were analyzed for changes in N-Myc or TRAP1 mRNA expression, by qPCR. Mean±SD (n=3). **, P=0.001. *D,* Schematic diagram of a human TRAP1 *promoter*. The position of putative c-Myc or N-Myc binding sites is indicated. ATG, translational initiation codon. E1, exon 1. *E,* P493 or PC3 cells expressing a wild type TRAP1 promoter (-1330 to +66; ~1.4 kb) upstream of a luciferase *reporter* gene (Luc-TRAP1) were transfected with the indicated siRNA and analyzed for luciferase activity. AU, arbitrary units. Mean±SD (n=6-11). ***, p<0.0001. *F,* The conditions are as in (*E*) and PC3 cells expressing WT Luc-TRAP1 or mutant Luc-TRAP1 (-60 Mut) cDNA were analyzed for

luciferase activity after transfection with control or Myc-directed siRNA. AU, arbitrary units. Mean±SD (n=9). ***, $p<0.0001$.



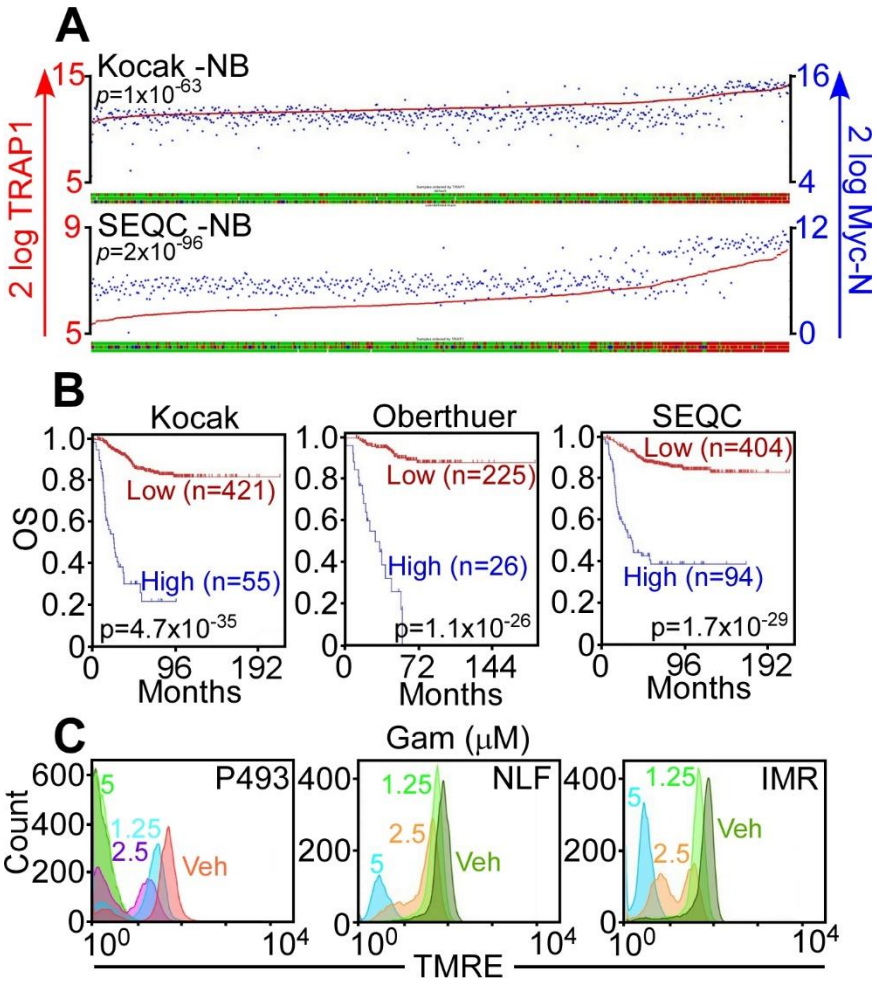
Supplementary Figure S5. Myc-TRAP1 regulation of mitochondrial protein folding and oxidative bioenergetics. *A*, PC3 cells transfected with siCtrl or siMyc were reconstituted with TRAP1 and detergent (CHAPS)-insoluble mitochondrial extracts were analyzed by Western blotting. The position of individual mitochondrial oxidative phosphorylation complex (C) subunits is indicated. Left panel, fully solubilized mitochondrial extracts. Representative experiment (n=2). *B and C,* The reconstitution conditions are as in (*A*) and transfected PC3 cells were analyzed for C. II function (*B*, representative tracings) and citrate synthase-normalized activity was quantified (*C*). Mean±SD (n=3). ***, p=0.0003 - <0.0001. *D and E*, The conditions

are as in (*B and C*) and transfected PC3 cells were analyzed for mitochondrial C. IV function (*D*, representative tracings) and citrate synthase-normalized activity was quantified (*E*). Mean±SD (n=3). *, p=0.01; **, p=0.001.



Supplementary Figure S6. Myc-TRAP1 regulation of tumor cell motility. *A,* PC3 cells transfected with siCtrl or two independent Myc-directed siRNA (siMyc #1 and siMyc #2) were reconstituted with TRAP1 cDNA and analyzed for cell motility in 2D contour plots. Each tracing corresponds to the movement of an individual cell. The siCtrl panel is the same as siCtrl in Fig. 3A. *B,* The conditions are as in (B) and the speed of cell motility (top panel) and total distance traveled by individual cells (bottom panel) was quantified. Mean±SD (n=20-21). ***, p<0.0001; ns, not significant. *C and D,* PC3 cells transfected and reconstituted as in (*A*) were analyzed for

invasion across Matrigel-coated inserts and DAPI stained nuclei of invaded cells (*C*, representative experiment) were quantified for each reconstitution condition tested (*D*). The siCtrl panel is the same as siCtrl in Fig. 3C. Scale bars, 50 μm. Mean±SD (n=10). **, p=0.001; ***, p<0.0001.



Supplementary Figure S7. Targeting Myc-TRAP1 for cancer therapy. *A,* Correlation between Myc and TRAP1 mRNA expression in two neuroblastoma (NB) patient cohorts (GEO 45547, Kocak, n=649; FDA-led Sequence Quality Control -SEQC consortium, n=498). Individual p values per each analysis are indicated. *B,* Kaplan-Meier overall survival (OS) curves of three neuroblastoma patient cohorts (Kocak, Oberthuer, SEQC) stratified for high *versus* low TRAP1 expression. The number of patients in each group is in parentheses. Individual p values per each analysis are shown. *C,* Burkitt lymphoma P493 or neuroblastoma NLF or IMR cells were treated with the indicated concentrations of mitochondrial-targeted, small molecule TRAP1 antagonist,

Gamitrinib (Gam, μM) and analyzed for mitochondrial membrane potential by TMRE staining and flow cytometry. Representative tracings (n=2).